REMARKS

The invention pertains to a method for increasing the specific activity of a mutated glycosyl hydrolase on a substrate relative to an unmutated form of the glycosyl hydrolase, by replacing an active site associated glycosyl-stabilizing amino acid of the hydrolase with an amino acid binding cellobiose less tightly than the glycosyl-stabilizing amino acid. The invention further provides glycosyl hydrolase variants and mutants of Y245G, Y42R, or W82R – all of which constitute cellulase enzymes characterized by improvement over the wild-type enzyme in the catalytic digestion of cellulose substrates. The increased catalytic activity is soluble and insoluble substrates.

The invention process, which led to creation of the specific mutant enzyme Y245G is applicable to create yet other mutant enzymes that have increased ability to solubilize cellulose, relative to their wild type counterparts. For instance, a number of glycohydrolases belonging to structural family 5 have been identified as being structurally analogous to E1 and as having specific residues, the aromatic side chains of which may perform functions equivalent to that of Tyr-245 in E1 (Table 1 of specification, left column). Mutation of these residues to the residues listed in corresponding rows of the middle column (Trp39 of 1A3H; Trp171 of 1BQC; Trp212 of 1CEN; Phe229 and/or Phe258 of 1CZ1; Trp259 and/or Trp811 of 1EDG; Trp30 of 2MAN) are reasonably to be expected, on the basis of computer modeling studies, to produce a decrease in the degree of product inhibition exhibited by the resulting mutant enzymes, relative to that exhibited by the wild-type enzymes, and as a result may also be expected to exhibit improved performance in the hydrolysis of cellulose.

Similarly, replacement of the residues listed in the right-hand column of Table I of the specification with residues having much less ability to form hydrogen bonds to the oxygen or hydrogen atoms of substrate hydroxyl groups can be expected to reduce the affinity of the enzyme active site for cellobiose. The mutant enzymes that may be produced in accordance with the present invention are exemplified by the examples in Table 1.

In view of the fact that no references have been cited in anticipation or obviousness of the invention – particularly as currently recited in the amended claims, it is believed that the application is now in condition for allowance, and it is respectfully requested that the foregoing discussion be factored into consideration upon taking the application up for examination.

Respectfully submitted,

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